



DEVELOPMENT OF STABILITY INDICATING, VALIDATED SINGLE DISSOLUTION METHOD FOR SIMULTANEOUS ESTIMATION OF CLOPIDOGREL BISULFATE AND RIVAROXABAN IN TABLET DOSAGE FORMULATION BY RP-HPLC METHOD

Rupali Sajjanwar (Rupali Jitendra Paranjape)*, Shyamala Bhaskaran, Kulesh Kakati, Shailendra Kumar Jha
Prist University, Thanjavur Road, Vallam, Tamil Nadu 613403

A dissolution method was developed for combination of two drugs which are already marketed as an individual product, Clopidogrel Bisulfate Tablets USP 75 mg and another is Rivaroxaban Tablets 10 mg & 20 mg. It was emphasized that both products have several advantages when given as combination therapy. Hence, the preference was given to develop a single dissolution method for the analysis of both the active components. This article presents a single dissolution method to accommodate both drugs. The method uses USP Type II Apparatus (paddles) at 75 rpm in 1000 mL of Acetate buffer (pH 4.5) medium containing 1% Sodium Lauryl Sulfate as surfactant at $37 \pm 0.5^\circ\text{C}$. This dissolution methodology provides good dissolution profiles for both Clopidogrel Bisulfate and Rivaroxaban and is able to discriminate the changes in composition, manufacturing process and stability for the combination tablets. To quantitate both drugs simultaneously, a rapid isocratic reversed-phase liquid chromatographic method was developed and validated.

Keywords: Dissolution, Method development, Validation, HPLC, Rivaroxaban, Clopidogrel Bisulfate, Tablet, Sink condition

INTRODUCTION

Clopidogrel Bisulfate Tablet is an adenosine diphosphate receptor inhibitor that prevents platelets in the blood from sticking together and forming clots [1, 2]. The empirical formula of Clopidogrel Bisulfate is $\text{C}_{16}\text{H}_{16}\text{Cl NO}_2\text{S}\cdot\text{H}_2\text{SO}_4$ and its molecular weight is 419.9. Clopidogrel Bisulfate, USP is a white to off-white powder. Clopidogrel Bisulfate tablets, contains 97.875 mg of Clopidogrel bisulfate which is the molar equivalent of 75 mg of Clopidogrel base [3,4]. Clopidogrel Bisulfate is fairly soluble and stable in aqueous solution at low pH, however solubility drops steeply when the solution of pH is above 3. Clopidogrel bisulfate exhibits poor dissolution in the pH range of 4.5 to 6.8 [5]. Rivaroxaban is used for prophylaxis of deep vein thrombosis (DVT), which may lead to pulmonary embolism (PE), in adults undergoing hip and knee replacement surgery as well as Rivaroxaban is used for stroke prophylaxis in patients with non-valvular atrial fibrillation [6, 7]. It is a white to yellowish powder with a molecular weight of 435.89. Rivaroxaban is practically insoluble in water and aqueous media with pH 1 – 9 (pH – independent, 5 – 7 mg/L are soluble at 25°C). It can be classified as a Class II substance in the Biopharmaceutics Classification System (low solubility, high permeability) [8]. The addition of very low dose anticoagulation with Rivaroxaban may represent a new

treatment strategy in patients with a recent acute coronary syndrome. A recent published trial found that a low dose Rivaroxaban to optimal antiplatelet therapy reduces mortality, cardiovascular mortality, infarct or stroke without significantly increasing fatal bleeding [9]. Hence it was decided to develop an in-vitro dissolution method for combination of Clopidogrel and Rivaroxaban Tablets for academic study purpose.

Dissolution is a physical test to predict the drug release from a dosage form for some precise time. Fundamentally, this process is controlled by the affinity between the solvent and the solid substance and the way by which the pharmaceutical system releases the drug. According to Mehta and coworkers dissolution test provides an indication of bioavailability of a drug and, thus, pharmaceutical equivalence from batch to batch. The dissolution test is an important tool in quality control of drugs and it becomes more important for drugs with relatively low water solubility [10, 11, 12].

FDA suggests that by using predictive mathematical model, the relationship between an in vitro property of the dosage forms and an in vivo response can be established. The concept behind establishing an IVIVC is that in vitro dissolution can serve as a surrogate for pharmacokinetic studies in humans, which may reduce the number of bioequivalence studies performed during the initial approval process e.g. a products

For Correspondence

paranjape_rupali@outlook.com

having multiple strengths as well as when certain scale-up and post-approval changes in the formulation need to be made[13].

A dissolution method should be discriminatory, and it should allow evaluating the performance of the product and possible changes it may suffer during routine commercial production and from stability studies. Many variables can influence the results of a dissolution test. Among these variables, we can find solubility, chemical nature of the drug, the dosage form, excipients and manufacturing technology employed, the apparatus used, the stirring speed, the use of devices for dosage forms (sinkers), the volume of media used, pH and temperature of the media, the filtration, and analytical method employed [14]. If the developed dissolution method mimics the GI-tract environment with discriminating power, it can be useful to forecast the *in vivo* behavior of formulations and to characterize the *in vitro* dissolution profiles of drug products during pharmaceutical development [15-19].

The Biopharmaceutics Classification System groups drugs into four classes. The class II category, low solubility and high permeability drugs are identified as potential drug candidates for investigation. The low solubility aspects can be handled in order to develop a bio-relevant and discriminating method for dissolution [20-24]. The development of a meaningful dissolution procedure for drug products with limited water solubility has been a challenge to both the pharmaceutical industry and the regulatory agencies [25]. Clopidogrel belongs to Class II (low solubility/high permeability), and its absorption in the gastrointestinal tract might be limited by the dissolution rate [26, 27, 28]. Compounds belonging to Class II are eligible to establish a significant *in vitro/in vivo* correlation (IVIVC), hence the appropriate selection for dissolution study's conditions is essential to have a method able to discriminate between products with potential problems of bioavailability. *In vitro* dissolution study is an official test of pharmacopoeias for evaluation of drug release from solid and semisolid dosage forms and to establish *in vitro/in vivo* correlation. It is also routinely used tool in Quality Control (QC) to ensure batch to batch consistency and in Research and Development (R&D) to provide some predictive estimate of the drug release in respect to the *In vivo* performance. When dissolution test is not defined in the monograph of the dosage form, or if the monograph is not available, comparison of drug dissolution profiles is recommended in dissolution media, in the pH range of 1.2–7.5. Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium. In this

study, the first approach was to compare buffers of different pH, as commonly used for solid dosage forms. The stirring speed selection was done based on the range recommended (50–75 rpm) for apparatus 2

Different analytical methods like colorimetric method, RP HPLC method and bio analytical method for estimation of Rivaroxaban [29-32]. Several spectrophotometric and high performance liquid chromatographic (HPLC) methods have been reported for the separation and quantitation of Clopidogrel or Pravastatin in biological fluids and drug product [33-37].

The present investigation was taken up to develop a suitable dissolution profile for the Rivaroxaban and Clopidogrel tablets combination in its dosage form, estimate and validate it by using RP-HPLC methods.

For immediate release products, dissolution method should confirm to one of the several methods currently specified for the dissolution requirement in USP. The volume of the dissolution medium is generally 500, 900, or 1000 ml. Sink conditions are desirable but not mandatory [13]. Actually the volume of dissolution medium to be used is defined considering "sink condition." The solubility of the drug substance is quantitatively determined in several dissolution media within the physiological pH range at 37 °C. Using this value, the volume of dissolution medium necessary to obtain a saturated solution of the highest dose of the product to be marketed is calculated. Sink condition is considered as at least 3 times this volume. Some companies work with 5 times or 10 times this value. There are some instances where the dissolution test is more discriminative if sink condition is not followed.[41].

For water insoluble or sparingly water soluble drug products, use of a surfactant such as sodium lauryl sulfate is recommended[38]. Due to the poor solubility of Rivaroxaban in water, using a surfactant such as sodium lauryl sulfate is essential[32].

Typical specification should range from 70 to 85 % at dissolution times between 30 and 60 minutes, specifications in excess of 85% are inappropriate since allowance must be made for assay and content uniformity of the formulation [39]. As per USFDA guideline, for poorly water soluble drug at two-point dissolution study, one at 15 minutes and the other at a later point (30, 45 or 60 minutes) to ensure 85 % dissolution is recommended [13]. The dissolution specification is expressed as the quantity Q of the active substance as a percentage of the content stated on the product label, which is dissolved in a specified time frame. As per British Pharmacopoeia unless

otherwise specified, the value of Q is 75 percent. In most cases when tested under reasonable and justified test conditions at least 75 % of the active substance is released within 45 minutes. Typically, one limit is specified to ensure that most of the active substances are dissolved within the preset time period[4].

MATERIALS AND METHODS

A HPLC SPD- 20AT (Shimadzu) equipped with detector SPD-20AT, pump LC-20AT, injector: Rheodyne injector (20 μ l Capacity), syringe: Hamilton (25 μ l) and chromatographic software: Spinchrom was used for the study purpose. Other equipments used for the study are pH Meter from Chemiline, India, Ultrasonicsonicator from Toshcon, Toshniwal process instrument pvt. Ltd. Ajmer, Analytical Balance: AX 200 etc. Manual Dissolution Apparatus Electro lab

Preparation of Dissolution Medium:

Dissolve 2.99g of Sodium acetate trihydrate to 1000mL of water. Adjust the pH to 4.5 ± 0.05 with glacial acetic acid. Add 10 gm Sodium lauryl sulfate to this buffer and mix well.

Dissolution conditions

Dissolution Medium: pH 4.5 Acetate buffer with 1.0% SLS

Medium Volume: 1000ml

Agitation: 75 RPM

Apparatus : USP TypeII (Paddle)

Time Point: 60 minutes

Temperature: $37.5 \text{ C} \pm 0.5 \text{ }^{\circ}\text{C}$

Withdrawal volume: 10ml

Chromatographic conditions:

Column: BDS hypersil C_{18} , 250mm \times 4.6mm, 5 μ (particle size), Thermo scientific

Flow rate : 1.0ml/min

Injection volume: 20 μ

Column Oven temp: Room Temp

Wavelength: 220nm

Run Time: 10 minutes

Preparation of mobile phase:

It is the mixture of buffer and methanol in ratio of 30:70.

Preparation of Buffer for mobile phase:

Buffer 0.05M potassium di hydrogen ortho phosphate buffer pH 4.0.

Dissolve 6.8gm potassium di hydrogen ortho phosphate buffer in 800ml water and dissolve. Adjust pH 4.0 of this solution

with 1% ortho phosphoric acid (H_3PO_4) and make up volume up to 1000ml with water.

Mobile phase:

Mix Buffer 0.05M potassium di hydrogen ortho phosphate buffer pH 4.0 and methanol in ratio 30:70.

Preparation of standard solution for dissolution:

Standard Stock Preparation:

Take 22mg Rivaroxaban working standard and 97.8mg Clopidogrel Bisulfate working standard into a 100 ml flask. In this flask 20ml methanol is added and sonicated for 10 minutes. Once the standards are dissolved make up the volume up to 100ml with methanol.

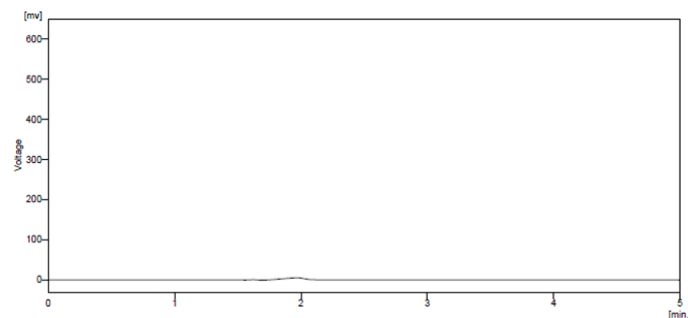
Standard preparation:

Further take 10ml from this solution to a 100ml volumetric flask. Make up with dissolution medium. (use this solution for std) (22ppm Rivaroxaban, 97.8ppm Clopidogrel Bisulfate)

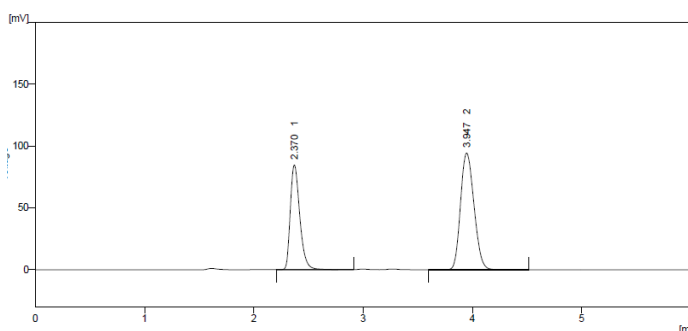
Preparation of Test solution for dissolution:

Transfer one tablet in each of the dissolution vessel containing dissolution medium pre-equilibrated to $37.5 \text{ C} \pm 0.5 \text{ }^{\circ}\text{C}$. Run the dissolution apparatus as per the above dissolution conditions. At specified time interval, withdraw 10ml of the sample aliquot from each of the dissolution vessel and replace with fresh dissolution media. Filter the sample aliquot through Whatman filter paper one, discarding few ml of filtrate, inject the sample into chromatographic system.

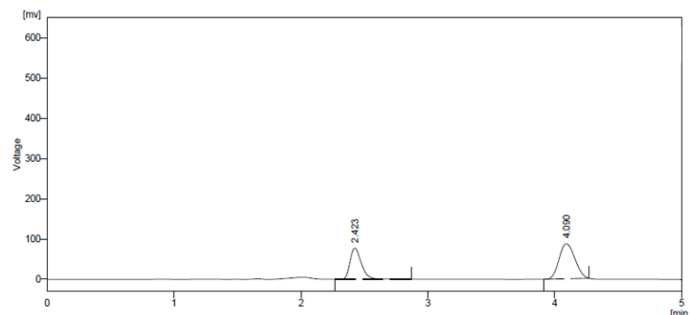
Chromatogram of Placebo



Chromatogram of System Suitability Solution



Chromatogram of Test Solution



RESULT & DISCUSSION

From the dissolution studies, it was observed that solubility of Rivaroxaban is very low at all physiological pH range 1.2 to 6.8; example in dissolution medium 0.1N HCl pH 1.2, Acetate buffer pH 4.5 and Phosphate Buffer pH 6.8. Rivaroxaban was not released completely in all the range tested, it also showed pH independent release profile. Whereas Clopidogrel Bisulfate released more than 90 % in acidic pH but it has less dissolution profile at pH 4.5 and pH 6.8 Phosphate buffer. Hence it was desirable to increase the solubility of both the drug at one physiological pH condition to get complete dissolution profile. We have selected pH 4.5 Acetate buffer for further study. To increase the solubility, it is decided to add surfactant in the dissolution medium.

It is observed that with 0.2 % SLS and 0.5 % SLS at 75 rpm drug release was less for Clopidogrel Bisulfate. At 1% SLS with 50 rpm dissolution was satisfactory but small variation was observed for individual unit, at initial stage Relative Standard Deviation (RSD) was found higher with 50 rpm. This may be due to less hydrodynamic properties of the dissolution medium at 50 rpm. It was well mentioned that at 50 rpm there is a chance of variation for a few products. Hence with 1 % SLS at 75rpm dissolution was carried out and found that more than 90 % drug release was observed in 60 minutes and sink condition is achieved. Based on the results obtained above, it was selected as discriminative dissolution test conditions for Rivaroxaban and Clopidogrel tablet combination and validated. The proposed HPLC method was simple, accurate, and reproducible for routine analysis of Clopidogrel and Rivaroxaban in dissolution test medium and in dosage forms. The results of this study showed that Rivaroxaban is unstable in almost all stress degradation conditions. The proposed stability-indicating method could be used for the determination of Rivaroxaban and Clopidogrel in the presence of tablet excipients and also degradation products. Since there is no

official method for Rivaroxaban and Clopidogrel tablets combination, the developed method could be used for quality control purposes.

CONCLUSION:

The dissolution test developed and validated for Rivaroxaban and Clopidogrel tablets (20mg & 75 mg) and found satisfactory. The dissolution conditions for this combination were 1000ml of acetate buffer pH 4.5 with 1% SLS at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, USP Type II (paddle) apparatus, 75 rpm stirring speed & filtration with easily available Whatman filter paper 1. The drug delivery was higher than 90 % within 60 min for all evaluated samples/products fulfilling the pharmacopeial guidelines for conventional solid oral tablet dosage form. More over this condition satisfied the criteria of drug stability during dissolution study. This method was validated to ensure that proposed method is linear, precise, accurate, sensitive, robust and cost effective. This can be used for dissolution study of this combination product in routine quality control successfully.

REFERENCES

1. Jackson AK. Platelet ADP receptor antagonists: ticlopidine and clopidogrel. NCBI Best Pract. Res. Clin. Haematol 2004 , 17 (1), 55-64.
2. SanjeevaY, Sivakumar A, Sameer G. Navalgund. Physico-chemical studies on stability of Clopidogrel tablet formulations. Int J Pharm Bio Sci. 2012 Oct; 3(4): (P) 433 – 439
3. USP monograph. Clopidogrel Bisulfate USP 32-NF 27: (P) 1992
4. Pravin B. Cholke, Raihan Ahmed, S. Z. Chemate, K. R. Jadhav. Development and Validation of Spectrophotometric Method for Clopidogrel bisulfate in pure and in film coated tablet dosage form. Arch. Appl. Sci. Res. 2012; 4 (1):59-64;
5. Zongyun Huang, Ruben Lozano, Robert Francis, Anne-Françoise Aubry, Alyson Steckbeck, Denis O. Sciascia. Development of a Single in Vitro Dissolution Method for a combination Trilayer Tablet Formulation of Clopidogrel and Pravastatin. Dissolution Technologies. Feb 2011; 12-19.
6. Pinaz A. Kasad, K.S. Muralikrishna. "Bayer's Xarelto Approved in Canada" (Press release). Bayer. "Bayer's Novel Anticoagulant Xarelto now also Approved in the EU". Bayer.

7. Pinaz A. Kasad, K.S. Muralikrishna. Design and Validation of Dissolution Profile of Rivaroxaban by Using RP-HPLC Method in Dosage Form. Asian J. Pharm. Ana. 2013; Vol. 3: Issue 3:Pg 75-78
8. CHMP Assessment report for Xarelto, European Medicine Agency Evaluation of Medicines For Human Use: Doc .Ref.: EMEA/543519/2008
9. Fernandez D, Penela D, Heras M. Contribution of the new oral anticoagulant to the treatment of acute coronary syndrome. Med Clin (Barc). 2012 OCT; 139 Suppl 2 : 41-45
10. Josilene Chaves RuelaCorrêa, Cristina Duarte Vianna-Soares, Hérica Regina Nunes Salgado. Development and Validation of Dissolution Test for Fluconazole Capsules by HPLC and Derivative UV Spectrophotometry. Chromatography Research International. Volume 2012 (2012), Article ID 610427
11. Paulo Costa, Sousa Lobo JM. Influence of dissolution medium agitation on release profiles of sustained-release tablets. Drug Development and Industrial Pharmacy. 2001; vol. 27, Issue 8: 811–817
12. Mehta J, Patidar K, Patel V, Kshatri N, Vyas N. Development & validation of an in vitro dissolution method with HPLC analysis for misoprostol in formulated dosage form. Analytical Methods. 2010; vol. 2, no. 1: 72–75
13. FDA, Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, Food and Drug Administration, Rockville, MD, 1997
14. Marcolongo R., Dissolução de medicamentos: fundamentos, aplicações, aspectos regulatórios e perspectivas na área farmacêutica, Thesis - Drugs and Medicines Post Graduation Program, University of São Paulo, São Paulo, Brazil, June 2011.
15. The United States Pharmacopoeia, Pharmacopeial Forum, 2004, 30, 351–363
16. Jigar Mehta, Kanhaiyalal Patidar, Vipul Patel, Nayan K Shatri, Niranjan Vyas. Development & validation of an in vitro dissolution method with HPLC analysis for misoprostol in formulated dosage form. Anal. Methods. 2010; Vol 2, no. 1: 72–75
17. Lagace M, Gravelle L, Di Maso M, McClintock S. Developing a discriminating dissolution procedure for a dual active pharmaceutical product with unique solubility characteristics. Dissolution Technologies. 2004; 2: 13-7
18. Soni T, Nagda C, Gandhi T, Chotai NP. Development of discriminating method for dissolution of Aceclofenac marketed formulations. Dissolution Technologies. 2008; 5: 31-35
19. Panikumar AD, Venkat Raju Y, Sunitha G, Sathesh B PR, Subrahmanyam CVS. Development of biorelevant and discriminating dissolution media for Efavirenz and its formulations. Asian J Pharm Clin Res. 2012; 5, Suppl 3: 220-223
20. Qingxi W, Nikolett F, Yun M. Biorelevant dissolution methodology and application in drug development. Dissolut Technol. Aug 2009; 6-12
21. Zoeller T, Klein S. Simplified biorelevant media for screening dissolution performance of poorly soluble drugs. Dissolut Technol. 2007; 11: 8-13
22. Dressman J, Kramer J. Pharmaceutical dissolution Testing. 1st ed. London: Taylor and Francis group. 2007; 92-98
23. Rohrs BR. Dissolution method development of poorly soluble compounds. Dissolut Technol 2001; 8: 1-5
24. Tenhoor NC, Bakatselou V, Dressman J. Solubility of Mefenamic acid under simulated fed and fasted state conditions. J Pharm Res 1991; 8: 1203-1205
25. Jogia H, Mehta T, Patel M. Evaluation of dissolution media containing a novel synthetic surfactant by in vitro testing of BCS class II drugs. Dissolut Technol 2009; 8: 14-19
26. Amidon GL, Lennernas H, VP Shah, J. R. Crison. A Theoretical Basis For a Biopharmaceutic Drug Classification. The Correlation of In Vitro Drug Product Dissolution and In Vivo Bioavailability. 1995; Pharmaceutical Research. Vol 12 no. 3: 413-420
27. Marc Lindenberg, Sabine Kopp, Jennifer B Dressman. Classification of orally administered drugs on the World Health Organization Model List of Essential Medicines according to the biopharmaceutics classification system. European Journal of Pharmaceutics and Biopharmaceutics. 2004; 58 (2): 265-278
28. Takagi T, Ramchandran C, Bermejo M, Gordon L Amidon. A provisional biopharmaceutical classification of the Top 200 oral drug products in United States, Great Britain, Spain and Japan. Molecular Pharmaceutics. 2006; 3(6): 631 – 643
29. Satyanarayana PVV, Alavala SM. New Spectrophotometric methods for the quantitative estimation of Rivaroxaban in formulations. International Journal of Research and Reviews in Pharmacy and Applied science; 2(4): 611-620

30. Harenberg J, Krämer R, Giese C, Marx S, Weiss C, Wehling M. Determination of Rivaroxaban by different factor Xa specific chromogenic substrate assays: reduction of inter assay variability. *Journal of Thrombosis and Thrombolysis*, J Thromb Thrombolysis 2011; 32 (3): 267–271
31. Satyanarayana PVV, Alavala SM, RP-HPLC method development and validation for the analysis of rivaroxaban in pharmaceutical dosage forms. 2012;2 (1): 226-231
32. Sour E, Mottaghi S. Development of a Stability-Indicating HPLC Method and a Dissolution Test for Rivaroxaban Dosage Forms. *Acta Chromatographica*. 2016; DOI: 10.1556/1326.
33. Pravin B C, Ahmed R. Development and Validation of Spectrophotometric Method for Clopidogrel bisulfate in pure and in film coated tablet dosage form. *Arch. Appl. Sci. Res.* 2012; 4 (1): 59-64
34. Singh SS, Sharma K, Barot D, Mohan P R, Lohray V B. Estimation of carboxylic acid metabolite of clopidogrel in Wistar rat plasma by HPLC and its application to a pharmacokinetic study. *J. Chromatogr. B Analyt Technol Biomed Life Sci.* 2005; 821 (2): 173–180
35. Ksycinska H, Rudzki P, Bukowska-Kiliszek M. Determination of Clopidogrel metabolite (SR26334) in human plasma by LC–MS. *J. Pharm. Biomed. Anal.* 2006; 41(2): 533–539
36. Gomez Y, Adams E, Hoogmartens J, Analysis of purity in 19 drug product tablets containing clopidogrel: 18 copies versus the original brand. *J. Pharm. & Biomed. Anal.* 2004; 34 (2), 341–348
37. Mitakos A, Panderi I. A validated LC method for the determination of Clopidogrel in pharmaceutical preparations. *J. Pharm. Biomed. Anal.* 2002; 28 (3–4): 431–438
38. Shah VP. In Vitro Dissolution Profile of Water Insoluble Drug Dosage Forms in the Presence of Surfactants. *Pharmaceutical Research*. 1989; 6: 612-618
39. Robert CW. Setting Dissolution Specifications. *Dissolution Technologies*. August 1997
40. Dissolution Test for Tablets and Capsules (Dissolution Test for Solid Dosage Forms) Ph.Eur. Appendix XII B.
41. William Brown, Margareth Marques. Question and Answer Section. *Dissolution Technologies*. May 2013

Received	11 th December 2014
Revised	19 th December 2014
Accepted	28 th December 2014
J. App. Pharm. Res., 3 (1); 2015: 26 – 31	